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ENHANCEMENT OF VIRAL TRANSFORMATION OF HAMSTER EMBRYO CELLS BY PRETREATMENT WITH 4-CHLOROMETHYLBIPHENYL*

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SUMMARY

Treatment of Syrian hamster embryo cells with 4-chloromethylbiphenyl produced reproducible and quantitative concentration-dependent enhancement of viral transformation the statistical significance of which was independent of cell lethality. These results suggest this compound has carcinogenic potential since positive results in this mammalian cell bioassay system closely correlate with the carcinogenic potential of many diverse compounds and with structurally similar agents, especially other chlorinated compounds.

Enhancement of virus transformation by physical or chemical agents has been successfully employed for over 10 years to assay for carcinogens using mammalian cells. Experimental data suggest that discrete changes in cell DNA resulting from chemical treatment can be successfully detected by observing a quantitative increase in viral transformation. Such increases in viral transformation have been demonstrated in hamster, rat and human cells treated with a variety of carcinogenic agents (Casto, 1981a). The viral enhancement assay reflects the capacity of a chemical to damage cell DNA by either direct or indirect means and provides a rapid mammalian cell bioassay system for screening of suspect genotoxic environmental chemicals. Concurrently, it has been shown that carcinogen treatment results in an

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increased incorporation of viral DNA into cellular DNA (Casto et al., 1979).

Data from bioassays of approx. 130 chemicals from 29 different chemical classes have been published using viral transformation enhancement assays. Unpublished or preliminary data exist for more than 100 other chemicals. These classes include alcohols and phenols, aliphatic amines, alkyl sulfates and sulfones, aromatic amines, aryl halides, carbohydrates and derivatives, hydroxylamines, mycotoxins, polycyclic hydrocarbons, hydrazines, inorganic metals, steroid hormones and chlorinated hydrocarbons (Casto, 1981a,b; Hatch et al., 1981a,b). The last 4 classes are generally negative in standard bacterial (Salmonella) assays (McCann et al., 1975; McCann and Ames, 1976).

Using the enhancement of adenovirus transformation as an index of carcinogenicity, 94% of 130 chemicals with known positive or negative activity agreed with their current in vivo classification (Casto, 1981a,b).

4-Chloromethylbiphenyl, a synthetic analog of 4-aminobiphenyl, has been recently reported to be a direct-acting bacterial mutagen and potential carcinogen (Ashby et al., 1981). The activity of this agent in BHK-21 cells, a continuous cell line derived from hamster kidneys, prompted an investigation of the ability of 4-chloromethylbiphenyl to enhance viral transformation in primary Syrian hamster embryo (SHE) cells. The viral enhancement assay has proven successful in detecting the potential carcinogenic activity of the related halogenated methanes; chloromethane and dichloromethane. This report documents the positive response of 4-chloromethylbiphenyl in this mammalian cell bioassay system.

MATERIALS AND METHODS

Transformation assays

Fresh stock solutions (50 mg/ml) of 4-chloromethylbiphenyl* were prepared for each experiment by dissolving the chemical in acetone. Appropriate dilutions were then made in complete culture medium to give the desired final concentration. 2 dishes of SHE cells were treated at each test dose for approx. 20 h. After pretreatments, SHE cells were rinsed with complete medium and inoculated with SA7. Adenovirus transformation procedures have been previously reported (Casto, 1968, 1973). SA7 virus was adsorbed for 3 h ($3–4 \times 10^7$ SA7 plaque-forming units per culture). The virus-inoculated cells were removed with trypsin (0.25% trypsin in modified Dulbecco's medium (MDM) with 0.1 mM $CaCl_2$), centrifuged, and resuspended to 10^6 cells/ml in MDM with 10% fetal bovine serum (FBS) and $NaHCO_3$ (0.11 g/100 ml). The cells were mixed vigorously and plated into 60-mm dishes using 2×10^5 cells/dish; 3 ml of the above medium were then added to each plate.

*Supplied by Dr. John Ashby, ICI, Ltd., Macclesfield of Cheshire (Great Britain).

After incubation for 3 days, the medium was changed to MDM with 1.0 mM $CaCl_2$ (Freeman et al., 1967; Casto, 1968), 10% FBS and $NaHCO_3$ (0.22 g/100 ml). After 6 days, transformation-assay plates were overlaid with 3 ml of the above medium containing Bactoagar (0.3 g/100 ml). At intervals of 4, 5 and 6 days, 3 ml of additional agar medium were added. Final focus counts were made 25–30 days from the beginning of the experiment.

Survival assays

After resuspension of the SHE cells to 10^6 cells/ml (see 'Transformation assays') the cells were diluted 1:300. 0.2 ml (666 cells) were then added to each of the 5 plastic dishes, followed by 3 ml of MDM with 10% FBS and $NaHCO_3$ (0.11 g/100 ml). 5–6 days later, 3 ml of medium with twice the amount of $NaHCO_3$ were added to each plate, and after 8–9 days of total incubation, the cell colonies were fixed in 10% buffered formalin and stained with 0.02% crystal violet. The cloning efficiency of virus-inoculated cells under these conditions was usually 8–12%. The number of colonies in 5 plates seeded with treated cells was divided by the number of colonies in 5 plates seeded with control cells to find the surviving fraction of treated cells.

Determination of enhancement

The total number of SA7 foci in 5 control plates, each receiving 2×10^5 cells, was used to determine the frequency of transformation per 10^6 virus-inoculated control cells. The transformation frequency (TF) of treated cells (number of SA7 foci per 10^6 surviving cells) was calculated by multiplying the number of SA7 foci from 5 plates by the reciprocal of the surviving fraction of treated cells. Detailed methods for determining the frequency of transformation and enhancement ratios using data from actual enhancement experiments have been presented (Casto et al., 1976, 1978). Similar methods for calculation have been used for the determination of mutation frequency (Chu, 1971) and for the demonstration of enhancement of SV40 transformation by DNA-base analogs (Todaro and Green, 1964) in mammalian cells.

Enhancement was expressed as the ratio between the TF of treated, surviving cells and the TF of control cells. Statistical significance was determined using a table of ratios (Casto et al., 1978) derived from the Lorenz table (Lorenz, 1962) that is based upon the Poisson distribution. The increased TF was considered statistically significant at the 5% or 1% confidence level if the enhancement ratio exceeded the appropriate value obtained from the Lorenz table.

RESULTS AND DISCUSSION

4-Chloromethylbiphenyl was bioassayed using 2-fold chemical dilutions extending over a 33-fold range (Table 1). The highest treatment concentration (50

TABLE 1

ENHANCEMENT OF VIRAL TRANSFORMATION OF HAMSTER EMBRYO CELLS BY PRETREATMENT WITH 4-CHLOROMETHYLBIPHENYL

Treatment dose (μg/ml)	Surviving fraction	SA7 foci[a]	Enhancement ratio[b]
50.0	62	247	2.8
25.0	99	208	1.5
12.5	93	135	1.1
6.3	93	143	1.1
3.2	101	159	1.1
1.5	102	136	1.1
0	100	144	1.0

[a]Number of foci from 10^6 plated cells.
[b]Solvent control, acetone, at an 0.5% final concentration.

μg/ml) produced significant toxicity (62% survival) as shown in the survival assay. Lower doses had no significant cytotoxic effect. Chemical pretreatment enhanced the viral transformation frequency in a concentration dependent manner. The 2 highest treatment concentrations (50 and 25.0 μg/ml) produced significantly increased enhancement ratios (2.8 and 1.5, respectively). A large absolute increase in viral foci, the statistical significance of which was independent of cell killing, is seen at both 50 and 25 μg/ml. Additional experiments utilizing the same treatment protocol but different lots of pooled hamster embryos are in close agreement with and confirm the data presented in Table 1.

Treatment of SHE cells with 4-chloromethylbiphenyl produced a concentration-dependent enhancement of viral transformation the significance of which was independent of cytotoxicity. These results suggest this compound has carcinogenic potential since positive results in this mammalian cell bioassay system closely correlate with the carcinogenic potential of many diverse compounds and with structurally similar agents, including chloromethane, dichloromethane, chloromethylmethylether, chlordane, 1,2-dichloroethane and 4,4-dichlorobenzidine (Casto, 1981a,b; Hatch et al., 1981a,b).

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